# ULTRASTRUCTURE AND HISTOCHEMICAL CHARACTERISTICS OF DERMAL ECCRINE CYLINDROMA (TURBAN TUMOR)*

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The dermal eccrine cylindroma (1), or so-called "turban tumor," has a particularly striking histologic picture. The cords of tumor cells are surrounded by a dense zone of hyaline material whose origin has been in dispute for many years (literature reviewed by Crain and Helwig (1), Evans (4), Gates *et al.* (5), and Ronchese (21)). The nature of the hyaline material cannot be understood without a knowledge of the site of origin of the tumor, and this too is in dispute (1, 4, 5, 21). In this paper we shall report our histochemical and electron microscopic observations of lesions from a patient with multiple dermal eccrine cylindroma, stressing the epithelial origin of the tumor and the possible nature and source of the associated hyaline material.

## MATERIALS AND METHODS

A detailed clinical history of the patient studied in this report has been presented elsewhere (6). The patient, a 59-year-old white female, noticed the appearance of tumors on the scalp at the age of about 37 years. When studied, the patient had numerous 1-10 mm. papular and nodular lesions scattered over the scalp, face, and neck. Initially, one nodule was removed and diagnosed by light microscopy as dermal eccrine cylindroma.

The material removed for the present study consisted of three nodules. The first had been present for 20 years (referred to as the "old" nodule); the second had been present for only 6 months (referred to as the "young" nodule); and the third had been treated 22 months prior to biopsy with 4,000 r of x-ray. The "old" nodule was fixed in Palade's buffered osmium tetroxide (19) and embedded in methacrylate. The "young" and irradiated nodules were fixed in Dalton's chrome-osmium fixative (2), and portions of the tissue were embedded in both methacrylate and in Epon 812 according to the method described by Luft (9).

The methacrylate-embedded tissue was cut into sections 1 $\mu$ thick with glass knives, mounted on glass slides, and stained by the methods outlined by Munger (14), including the hematoxylin and phloxine (H & P), periodic acid-Schiff (PAS), toluidine blue, and a modified Hale colloidal iron technic for acid mucopolysaccharides (13). Formalin-fixed tissue sections from the material used by Crain and Helwig (1) were also studied, and these were prepared by the following methods: hematoxylin and eosin (H & E), alcian blue, Hale colloidal iron, PAS, and aldehyde-fuchsin. Some sections of formalin-fixed tissue were treated with bovine testicular hyaluronidase for 1 hour prior to staining with alcian blue and colloidal iron. The procedures were carried out as outlined in the Manual of Histologic and Special Staining Technics (10). Thin sections of either the methacrylate- or Epon-embedded tissue were mounted on formvar-coated grids and examined in RCA EMU 3D and 3F electron microscopes. Some of the Epon-embedded sections were mounted directly on copper grids. Epon-embedded sections were examined with and without staining with uranyl acetate (24).

## OBSERVATIONS

### *"Young" Nodule—Present for Six Months*

*Light Microscopy:* Cords of tumor cells surrounded by prominent hyaline membranes are present in a loose connective tissue stroma as seen in osmium-fixed, methacrylate-embedded H & P-stained sections (Fig. 1). The appearance of such a section is comparable to those of the tumor prepared by conventional methods (1). The individual tumor cells have large basophilic nuclei with scant cytoplasm. The nuclei at the periphery of the cords have a more compact and dense, darker staining nucleoplasm. Prominent intercellular spaces are present between adjacent tumor cells in some areas (Fig. 2), whereas in other areas the cells are closely approximated. These two types of tumor cell growth are especially prominent in electron micrographs (see below)

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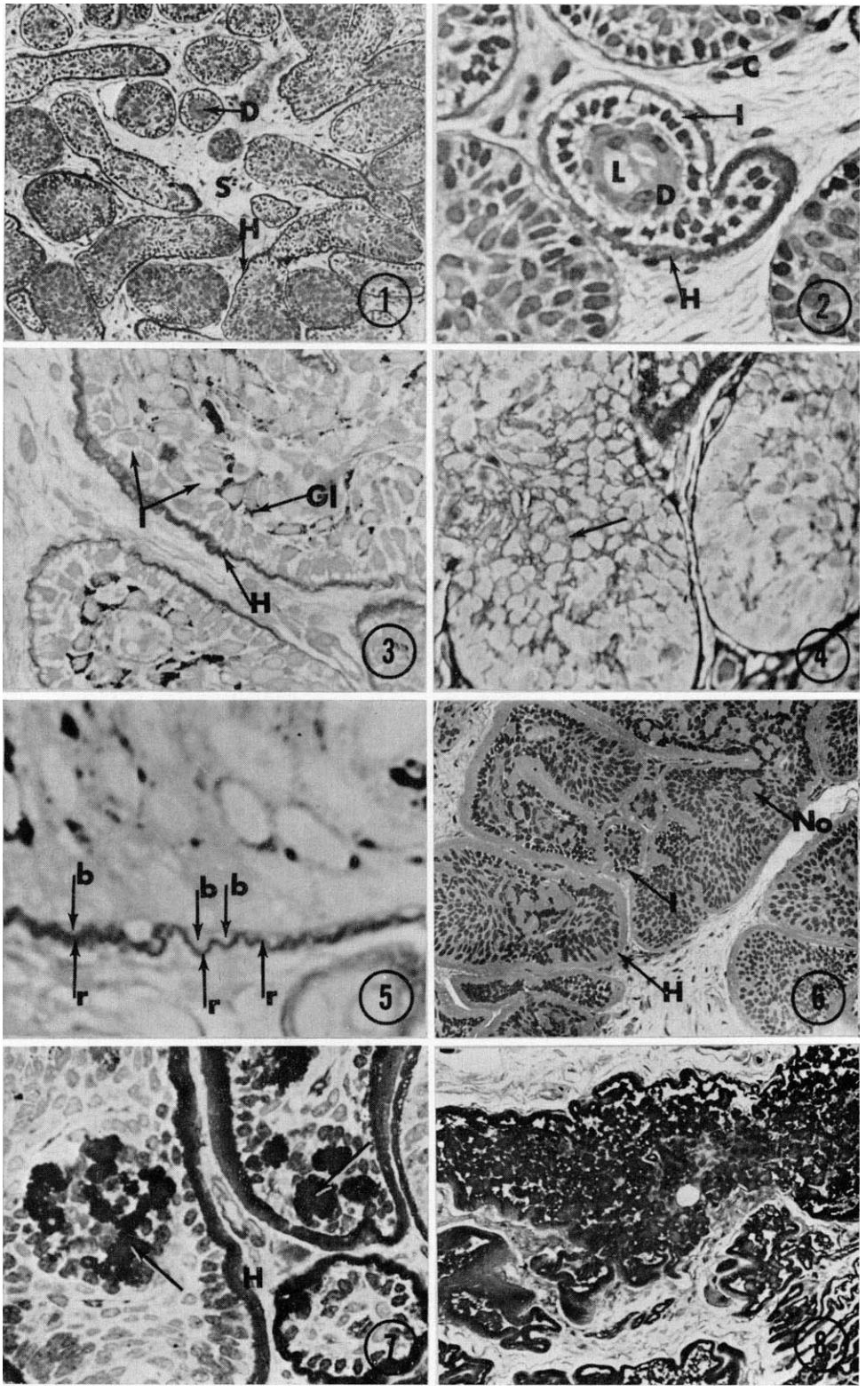


FIGS. 1 to 8

and are designated as loose (reticular) and solid (squamoid) patterns, the former having prominent intercellular spaces, the latter lacking intercellular spaces.

Surrounding the cords of tumor cells is a prominent eosinophilic hyaline membrane (Figs. 1 and 2) varying in thickness between 1 and 5 $\mu$ (microns). In some areas an undulating appearance of the hyaline material is suggested (Fig. 2). The hyaline material is interpreted on the basis of arguments presented in the discussion to represent basement membrane material, and will be so designated. The hyalinized basement membrane is intensely PAS positive and diastase resistant, which probably indicates the presence of a neutral mucopolysaccharide, mucoprotein, or glycoprotein (Fig. 3). In such preparations a definite undulation of the PAS-positive material is seen (Fig. 3). PAS-positive material is also present within some of the individual epithelial cells making up the tumor cords. This latter material is diastase labile, thereby identifying it as glycogen (Fig. 3).

In sections of osmium-fixed tissue prepared by the colloidal iron technic (Fig. 4), material in the intercellular spaces in cords of tumor cells is intensely reactive (blue color), indicating the presence of acid mucopolysaccharide. This material is PAS and aldehyde-fuchsin negative, and does not exhibit metachromasia with toluidine blue. Similar staining reactions are seen in formalin-fixed tissue. Portions of the hyalinized basement membrane are also reactive for acid mucopolysaccharides with colloidal iron (Fig. 4). When the PAS and colloidal iron technic are combined (Fig. 5), PAS demonstrates the undulating basement membrane, while the colloidal iron shows positive material within pockets formed by the undulations of the basement membrane (Fig. 5). In such a combined procedure, the acid mucopolysaccharide material in the intercellular spaces in the cords of cells is also demonstrated by the blue color (Fig. 4). On the outer side of the PAS-positive basement membrane is a zone of acid mucopolysaccharide material contiguous with the connective tissue surrounding the cords of tumor cells. In sections of formalin-fixed tissue prepared by the colloidal iron technic, prior digestion with testicular hyaluronidase did not remove the acid mucopolysaccharide material within the basement membrane and cords of tumor cells. The acid mucopolysaccharide material of the connective tissue stroma was digested, indicating that this acid mucopolysaccharide is probably hyaluronic acid.

Duct lumina are present within the cords of tumor cells, and these are lined by either cuboidal or flattened epithelial cells (Fig. 2). The zone of cellular cytoplasm adjoining the lumen is brightly eosinophilic and similar to the "cuticular border" of the eccrine sweat gland duct (11, 12). The lumina of these ductal structures usually contain granular eosinophilic material that is PAS positive and diastase resistant, and this probably indicates the presence of a neutral mucopolysaccharide, mucoprotein, or glycoprotein. The material is also reactive with colloidal iron, probably indicating the presence of an acid mucopolysaccharide.

No intralobular hyaline masses or droplets were observed.

FIG. 1. "Young" nodule. Osmium-fixed tissue, H & P-stained section. Cords of tumor cells are embedded in a loose stroma (S), and the individual cords are surrounded by a prominent eosinophilic hyaline membrane (H). Within the cords of cells duct-like structures (D) are present. × 180 (AFIP Neg. 60-6001-3).

FIG. 2. "Young" nodule. Osmium-fixed tissue, H & P-stained section. The tumor cells forming the cord in the center have prominent nuclei, and between adjacent cells are large intercellular spaces (I) of nonstaining material. A distinct duct-like structure (D) with a lumen (L) is surrounded by the loosely knit cells. At the lower right is a cord of cells that lack prominent intercellular spaces, *i.e.*, a "solid" pattern. The hyaline membrane (H) surrounds individual cords of tumor cells. At the upper right is a capillary (C) in the stroma adjacent to a tumor cord. × 485 (AFIP Neg. 60-6001-1).

FIG. 3. "Young" nodule. Osmium-fixed tissue, PAS-prepared section. The prominent hyaline membrane (H) seen in Figs. 1 and 2 is intensely PAS positive, as are accumulations of glycogen (G) within the tumor cells. The hyaline membrane appears to undulate as it surrounds the cord of tumor cells. The intercellular spaces (I) are prominent. × 485 (AFIP Neg. 60-6003).

FIG. 4. "Young" nodule. Osmium-fixed tissue, Hale colloidal iron-prepared section. Between the cells or in the intercellular spaces are accumulations of blue material (arrow), indicating the presence of acid mucopolysaccharide. The hyaline zone around the cords of tumor cells also contains acid mucopolysaccharide-positive material. × 485 (AFIP Neg. 60-6004).

FIG. 5. "Young" nodule. Osmium-fixed tissue, Hale colloidal iron- and PAS-prepared section. The PAS-positive basement membrane material is red (r) and is focally separated from the tumor cells by accumulations of acid mucopolysaccharide, which appears blue (b). The intercellular spaces between the tumor cells stain as in Fig. 4, but the photograph was taken to illustrate the red PAS-positive basement membrane. × 1,200 (AFIP Neg. 60-6005).

FIG. 6. "Old" nodule. Osmium-fixed tissue, H & P-stained section. The cords of tumor cells are surrounded by a very dense and thick hyaline membrane (H). Within the cords of tumor cells are nodules (No) of hyaline material, which in some areas appear to connect with the hyalinized basement membrane surrounding the cords of cells. Intercellular spaces (I) are present. × 180 (AFIP Neg. 60-5999).

FIG. 7. "Old" nodule. Osmium-fixed tissue, PAS-prepared section. The hyalinized basement membrane (H) surrounding the cords of cells is intensely PAS positive, as are the nodules (arrows) of hyaline material within the cords of cells. × 485 (AFIP Neg. 60-6000).

FIG. 8. Irradiated nodule. Osmium-fixed tissue, H & P-stained section. Undulating masses of hyalinized material are present, and only a few cells representing fibroblasts are seen. × 180 (AFIP Neg. 60-6002).


FIG. 9. Electron micrograph of a portion of one cord of tumor cells from the "young" nodule. The tumor cells have large, prominent nuclei (N) with scant cytoplasm that extend as finger-like projections to contact adjacent cells. Between the fingers of tumor cell cytoplasm are prominent intercellular spaces (I) containing a fine granular material. Between the points of contact of adjacent tumor cells are numerous desmosomes (D). Surrounding the cord of tumor cells is a zone of ill-defined material representing the hyalinized basement membrane (H) seen by light microscopy. Fibroblasts (F) and collagen (C) are present in the surrounding connective tissue stroma. × 4,200.

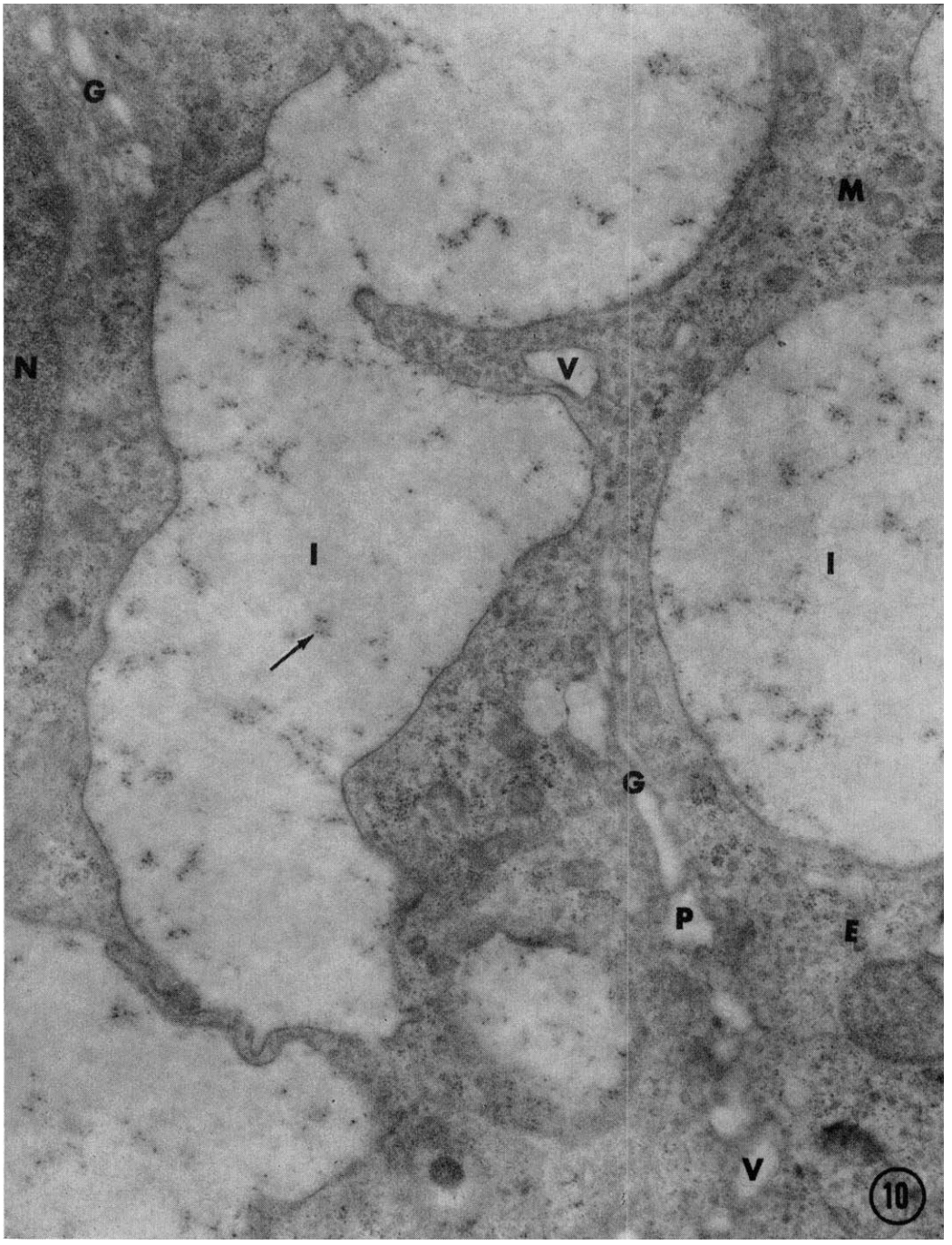


FIG. 10. A portion of the cytoplasm of one tumor cell from an area similar to that illustrated in Fig. 9. The nucleus (N) of this cell is irregularly lobulated. In the cytoplasm of the cell are scattered mitochondria (M) and numerous profiles of ergastoplasmic sacs (E), in addition to a background granularity of ribonucleoprotein particles and other granular material. The Golgi apparatus (G) of this cell is very large and is associated with several vacuoles, which may represent secretory vacuoles in the process of formation, that is, prosecretory vacuoles (P). Other vacuoles (V) are present that are considered to be mature secretory vacuoles. The intercellular space (I) contains small dense granules (arrow) that are always seen as round profiles in section. Vacuoles do not contain these dense granules and hence are not invaginations of the plasma membrane of the cell. Such a prominent Golgi apparatus, with its associated vacuoles, is similar to that seen in the mucoid cell of the eccrine sweat gland, and these vacuoles are thought to represent secretory vacuoles. × 22,500.

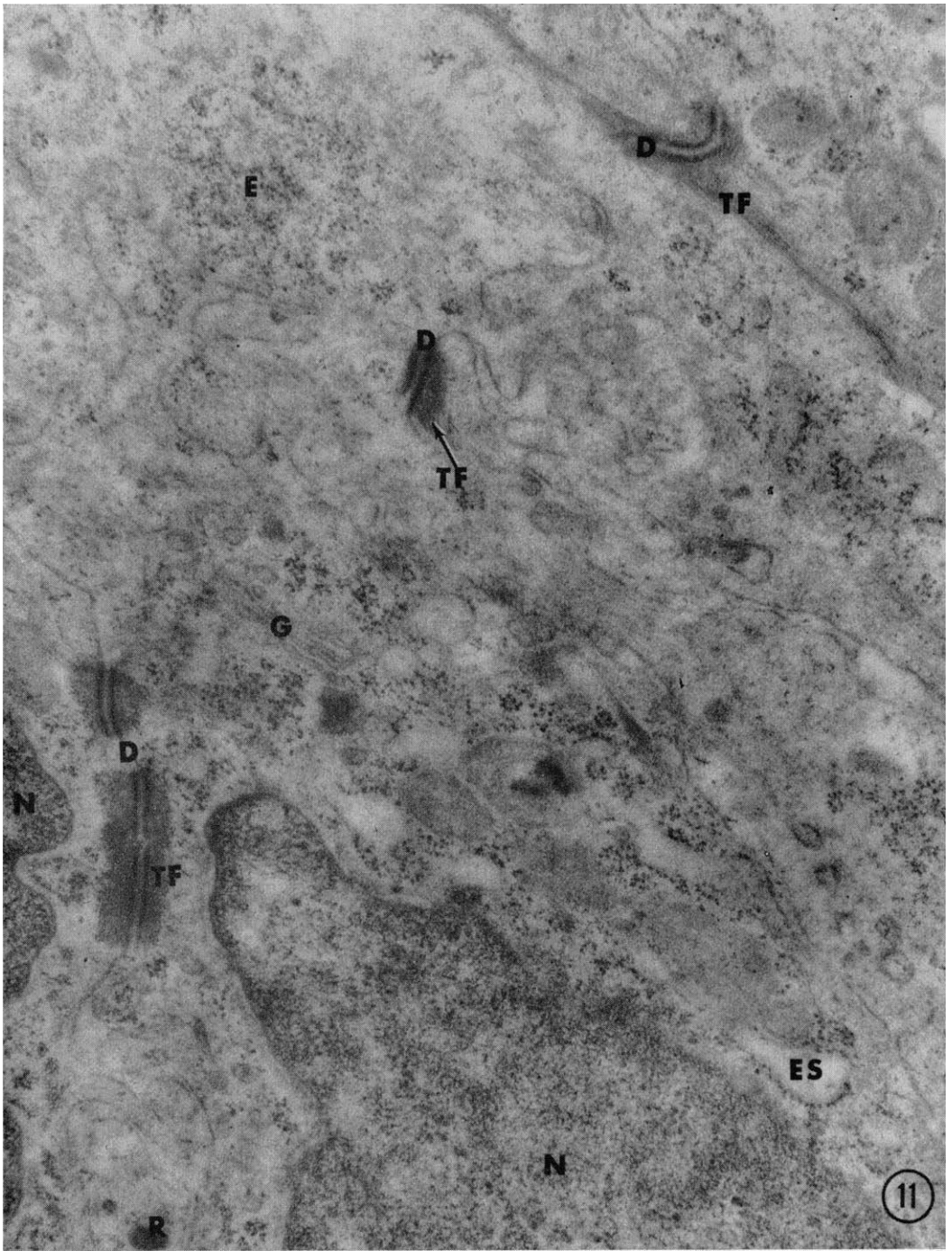


FIG. 11. Squamoid pattern of a cord of cells from the "young" nodule. The nuclei (N) of two adjacent cells are illustrated in this micrograph. Prominent desmosomes (D) connect adjacent cells. Those in the lower left illustrate tonofilaments (TF) inserting into the desmosome, cut in cross section; those in the center have the tonofilaments in partial tangential section, and at the upper right the tonofilaments are in longitudinal section. The ergastoplasm is represented both by the presence of ergastoplasmic sacs (ES) and free ribonucleoprotein granules (E). A small Golgi apparatus (G) is seen in the central cell. A dense granule (R) of unknown nature is present. × 26,125.

*Electron Microscopy:* The loose pattern of tumor cells is most prominent in this specimen (Figs. 9 and 10). Individual cells have large prominent nuclei, with scant finger-like strands of cytoplasm radiating from the nuclear area (Figs. 9 and 10). Between the fingers of cytoplasm are prominent intercellular spaces containing fibrillar and dense granular material (Fig. 10). Occasional desmosomes are present between contiguous fingers of adjacent cells (Fig. 9). Within the cytoplasm of the cells are numerous mitochondria, a few scattered sacs of ergastoplasm, occasional clumps of ribonucleoprotein granules, and in some cells a very prominent Golgi apparatus (Fig. 10). In cells with a prominent Golgi apparatus, large vacuoles containing fine fibrillar material are present in the cytoplasm. These vacuoles are not portions of invaginations of the plasma membrane, since the membrane bounding the vacuoles is single and the vacuoles do not contain the dense granules seen in the intercellular spaces (Fig. 10). These vacuoles appear to be very similar to the secretory vacuoles of the mucoid cell of the eccrine sweat gland (13, 16, 17) or to the mucous vacuoles of the goblet cell (20). On the basis of arguments presented in the discussion, they are considered to represent secretory vacuoles. Such secretory vacuoles always are in intimate relationship to the Golgi apparatus, where prosecretory vacuoles can be identified as transitional forms to mature secretory vacuoles.

The solid cellular areas of the tumor lack prominent intercellular spaces, and the cells are in direct apposition to one another, with little or no intervening intercellular space (Fig. 11). The ultrastructure of the cytoplasm of such cells appears to be identical to that of the reticular pattern cells, except that in general a prominent Golgi apparatus is not seen (Fig. 11). More of the squamoid cells are connected by desmosomes than are the reticular cells, and bands of tonofilaments can usually be seen coursing through the cytoplasm of the squamoid cells (Fig. 11). The desmosomes (Fig. 12) in this tumor have an ultrastructure identical to that of the desmosomes connecting cells of the eccrine sweat gland duct (3, 13, 16, 17) and those connecting cells of stratified squamous epithelium as described by Odland (18). Duct-like structures are always present and surrounded by solid groups of cells, as described in detail below.

Cells of both loose and solid patterns have occasional dense cytoplasmic granules, which are very prominent in some cells (Figs. 11, 13, 14). Such granules measure about 150 mμ in diameter

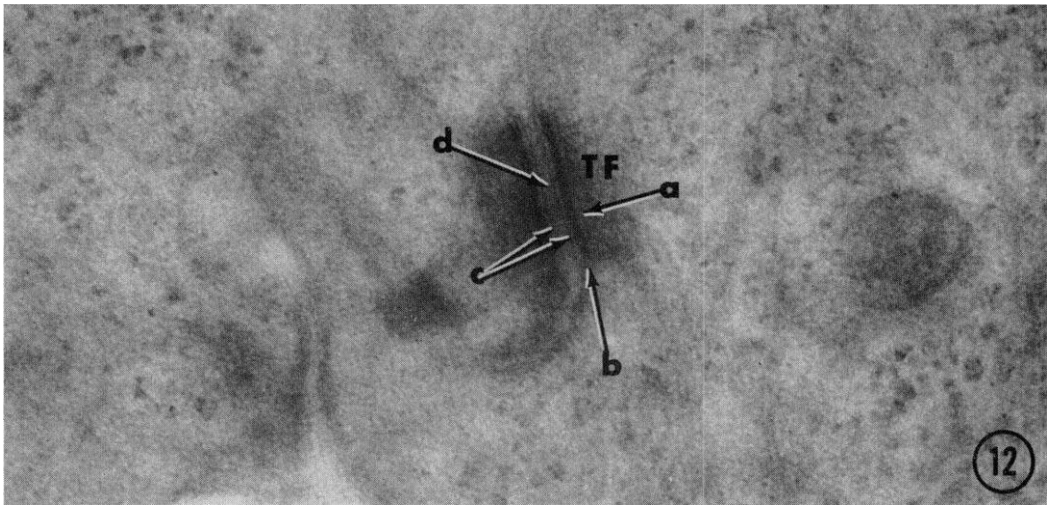


FIG. 12. "Young" nodule. A desmosome connecting two adjacent tumor cells. The desmosome consists of a dense zone on either side of the thickened plasma membranes (b) representing the attached tonofilaments (TF) in tangential section, which produce a discrete dense band (a) separated by a space from the plasma membranes. The plasma membranes of the adjacent cells are thickened and more dense than usual. Between the plasma membranes are three bands, the intermediate dense layers (c) and the intercellular contact layer (d) represented as an indistinct central dense band. × 80,465.

and have a dark central core separated from a definite limiting membrane by a less dense space. The nature and function of these granules is unknown, as is the case with similar granules seen in secretory cells of the eccrine sweat gland (16), where they were considered as possibly representing virus particles.

Cytoplasmic inclusions are present in some cells, and appear as large membranous or partially membranous vacuoles (Figs. 13 and 14). These latter inclusion vacuoles appear to be quite different from the mature secretory vacuoles (Fig. 10), which contain only faintly fibrillar material.

Surrounding the cords of cells is a basement membrane, which in some areas is delicate and well defined, but which in other areas appears as a massive confluence of poorly defined homogeneous material separated by less dense spaces associated with numerous collagen fibers (Figs. 13, 14, 15). When a massive accumulation of hyalinized material is present, the structure is exceedingly complex, whereas when the accumulations are minimal, it is easier to identify its structural components (Fig. 13). This basement membrane can be defined as a relatively electron-dense homogeneous band of material, which corresponds to the PAS-positive diastase-resistant material seen in the light microscope adjacent to the base of the tumor cells as they abut on

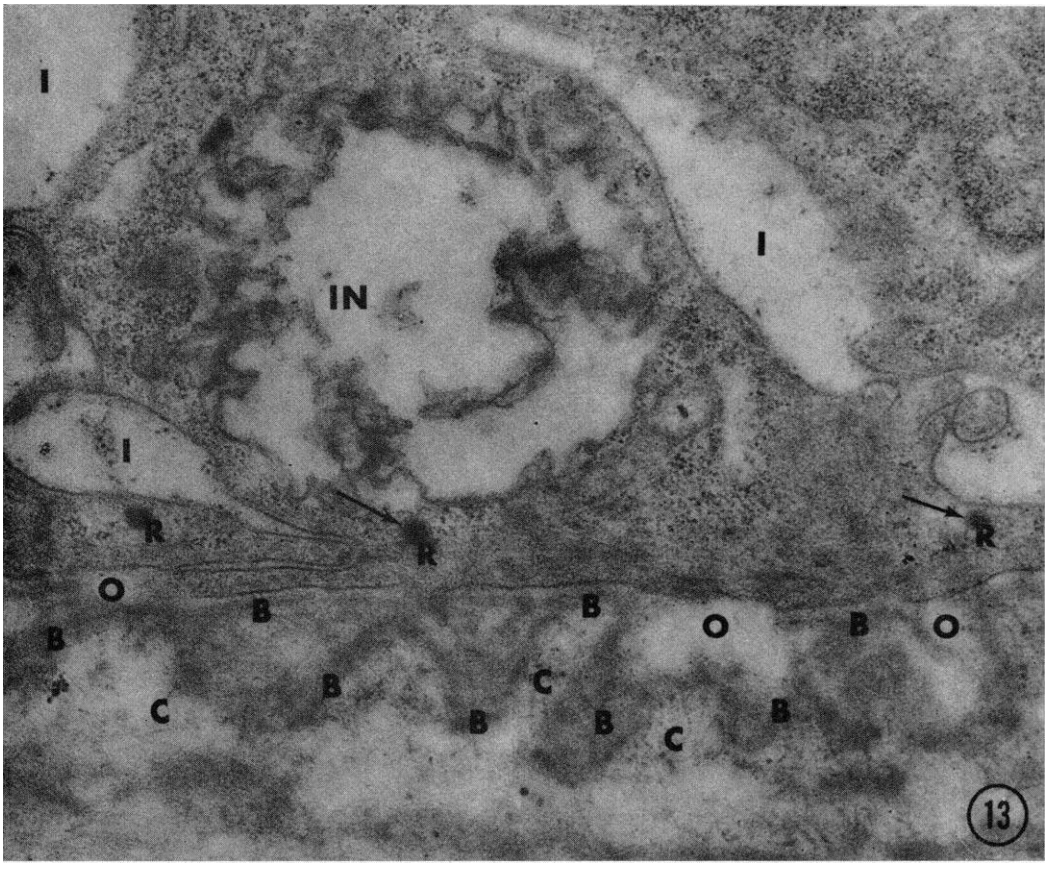


FIG. 13. "Young" nodule. The basal portion of a tumor cell abutting on a relatively thin hyalinized basement membrane. This electron micrograph shows a reticular area in the tumor having prominent intercellular spaces (I). Within one cell is a large, partially membranous inclusion (IN). Small, dense granules (R) with a definite limiting membrane (arrow) are present in the cytoplasm of two cells. The basement membrane (B) is a complex undulating, bandlike structure. In some areas a definite basement membrane (B) can be seen adjacent to the tumor cells, whereas in other areas this dense amorphous band appears to be separated from the base of the cells by empty spaces (O). Small collagen fibers (C) insert into the basement membrane on its external surface. × 33,250.

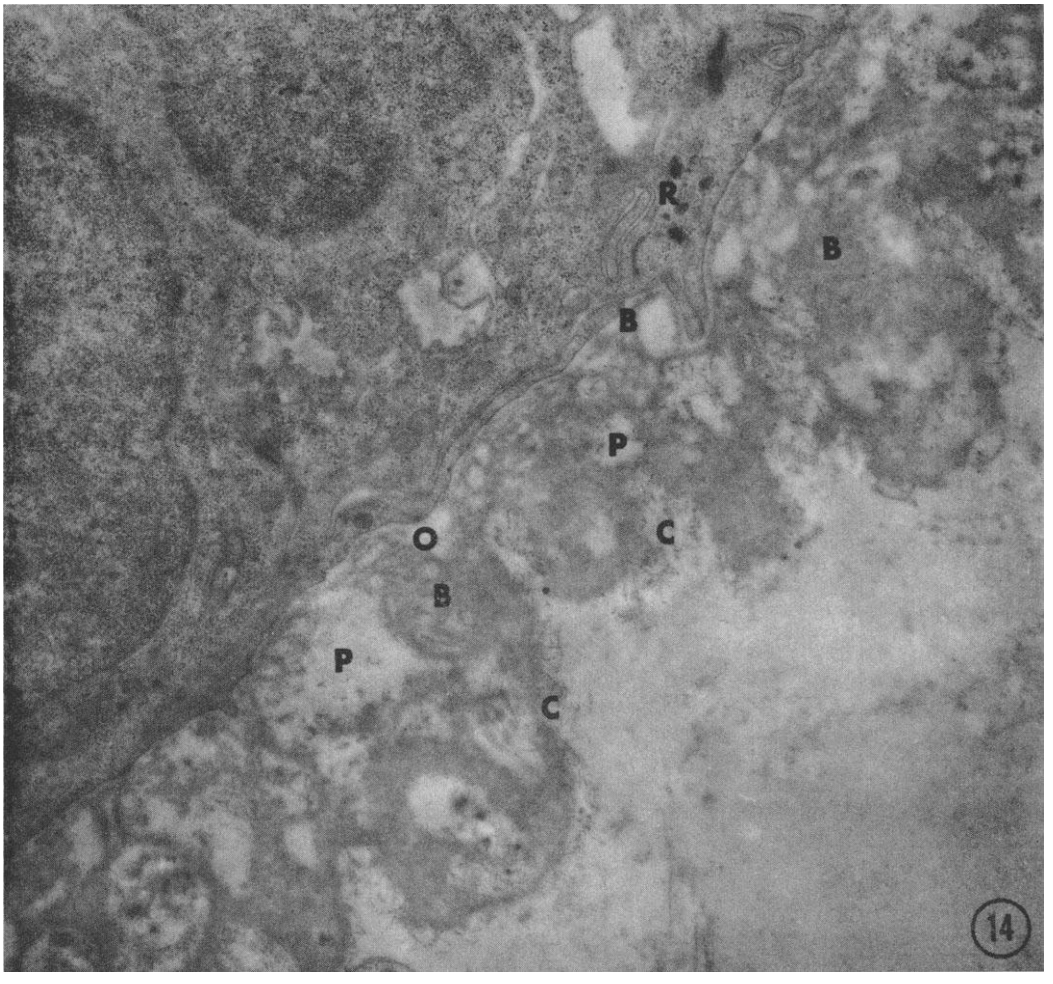


FIG. 14. Basal portion of tumor cells in area similar to Fig. 13, but the hyalinized basement membrane (B) is thicker and the cells are more squamoid in pattern. The cytoplasm of these cells contains dense granules (R) similar to those seen in Fig. 13. The basement membrane (B) can be traced as a dense, undulating homogeneous zone that in focal areas (O) has no contact with the tumor cells. Within the lobulated outline of the basement membrane are pockets (P) of less dense material, which correspond to the pockets of acid mucopolysaccharide seen in Fig. 5. Small collagen fibers (C) insert into the external surface of the basement membrane. × 16,625.

the connective tissue stroma (Fig. 3). In electron micrographs this dense band is usually separated from the plasma membrane of the epithelial cells by a narrow space or larger focal pockets of material (Fig. 13) of low electron density. These focal pockets correspond to the acid mucopolysaccharide material seen in Figure 5. Between some of the pockets of acid mucopolysaccharide material and the plasma membrane of the tumor cell, basement membranes are intact. Thus, two basement membranes (or more) are interposed between a tumor cell and the connective tissue stroma. The individual laminations of basement membranes are separated by pockets of acid mucopolysaccharide. The portion of the basement membrane that abuts on the connective tissue stroma can be easily identified, since collagen fibers insert into the external surface (Figs. 14 and 15). When the hyalinized basement membrane is prominent (Figs. 14 and 15), the pockets of acid mucopolysaccharide become more condensed and the major portion of the material appears as laminated excrescences of the basement membrane. Even in the thickest part of

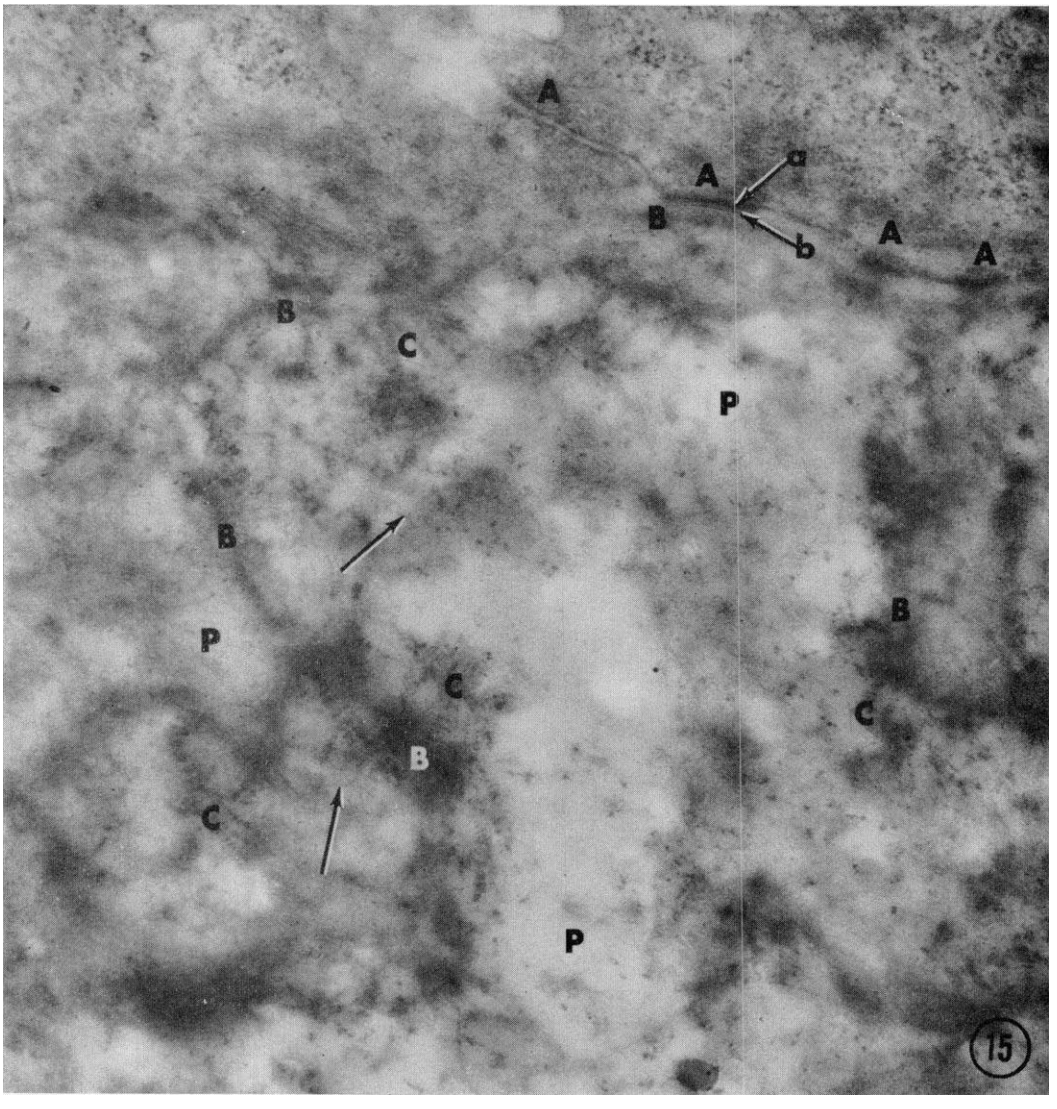


FIG. 15. "Young" nodule. Higher magnification of the prominent, thick hyalinized basement membrane for comparison with Figs. 13 and 14. The base of the tumor cell is seen at upper right, and numerous dermal attachment plates (A) are present. These dermal attachment plates consist of a thickened plasma membrane (a) and an intermediate dense band (b) between the plasma membrane and the basement membrane (dark B). The intermediate dense band (b) has no apparent connection with either the plasma membrane or the basement membrane. Within the hyalinized basement membrane there are large pockets of material (P) of low electron density and dark bands (light B) of high electron density into which collagen fibers (C) insert. The collagen fibers have a normal 529 Å periodicity. In the midst of the hyalinized material are numerous very small fibers whose nature is impossible to determine (arrows). × 35,600.

the hyalinized basement membrane, the collagen fibers maintain their original position of insertion into the stromal surface (Fig. 15). The hyalinized basement membrane is thus composed of basement membrane material, pockets of acid mucopolysaccharide probably secreted by the tumor cells, and the attachment of collagen on the stromal surface.

The basal plasma membrane of the tumor cells as they abut on the basement membrane area is focally specialized into dermal attachment plates (Fig. 15) similar to those seen in the eccrine

sweat gland duct (16) and in stratified squamous epithelium (22, 23). The plasma membrane is focally thickened, and tonofilaments insert into the specialized dermal attachment plates. Between the plasma membrane and the basement membrane is a distinct dense band. The general appearance of such attachment plates is basically similar to half of a desmosome (16, 18, 23).

Duct-like structures are present in the midst of the cords of tumor cells, and these appear similar to the eccrine sweat gland duct (3, 13, 16, 17). Two types of ducts are present (Fig. 16). In one type, the lumen contains a granular material and is lined by cuboidal epithelial cells, the apical portions of which are specialized into a "cuticular border" (Figs. 16 and 18). This "cuticular border" is composed of an accumulation of numerous tonofilaments and dense granules (Fig. 19) but is much less dense than the similar structures present in the normal eccrine sweat gland duct (13, 16, 17). The tonofilaments are associated with the numerous desmosomes connecting adjacent cells. The cuboidal cells lining the lumen lack intercellular

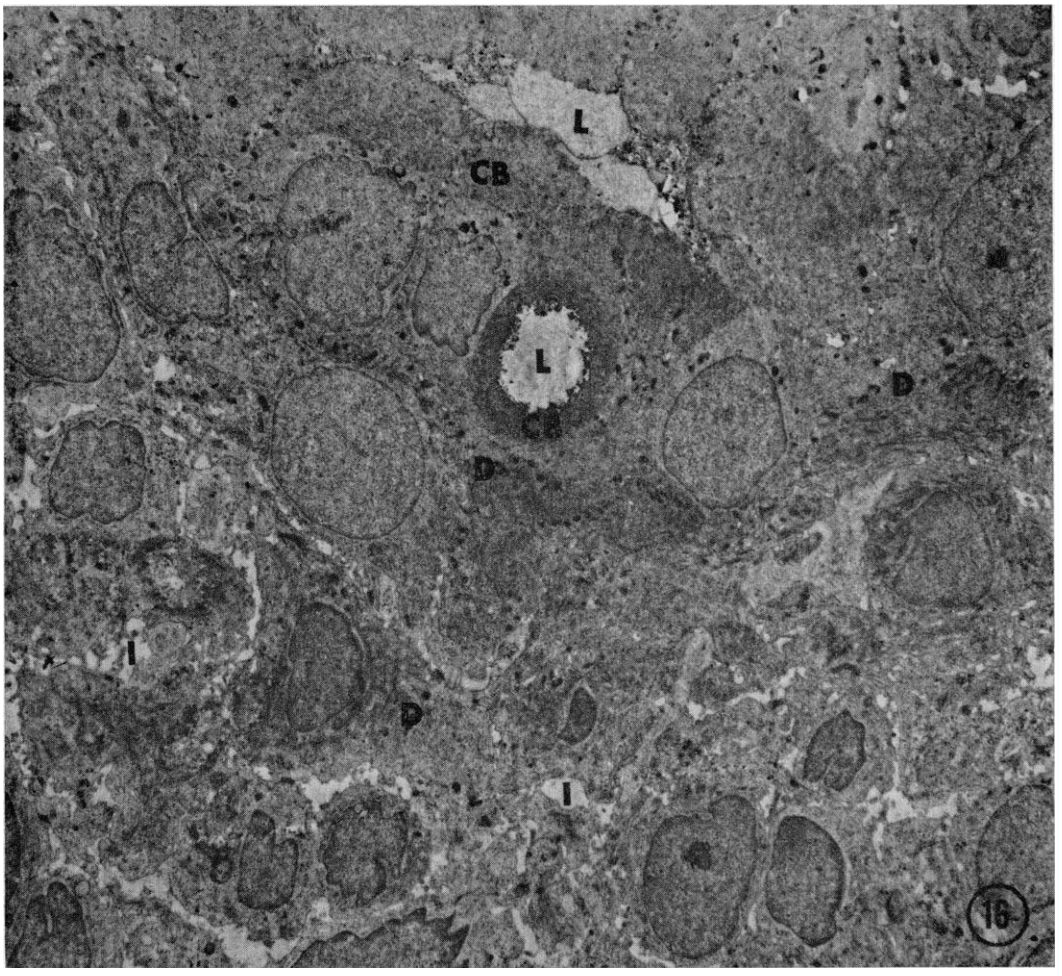


FIG. 16. "Young" nodule. A cord of tumor cells at low magnification illustrating two types of ducts. Each duct has a distinct lumen (L) bounded by a zone of cytoplasm called a "cuticular border" (CB) that is devoid of organelles such as mitochondria. The upper lumen contains membranous forms. The cells abutting on the duct lumen are squamoid in pattern, whereas the cells of the next surrounding layer are reticular in type and separated by prominent intercellular spaces (I). Numerous desmosomes (D) are especially concentrated in the squamoid portion of the cord of tumor cells surrounding and abutting on the duct lumens. × 3,600.

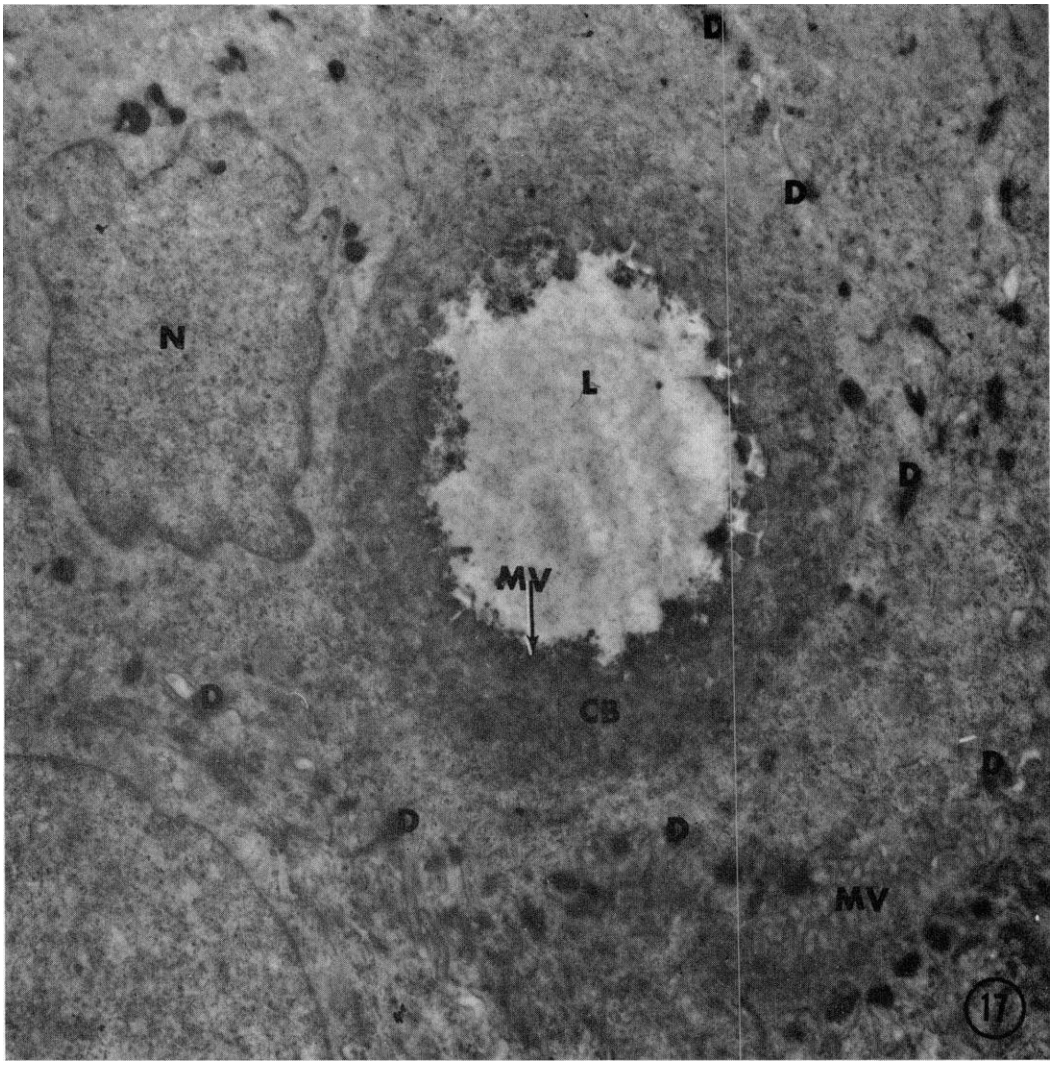


FIG. 17. Higher magnification of the smaller duct lumen illustrated in Fig. 16. The lumen (L) is bounded by a dense zone of cytoplasm that is similar in structure to the cuticular border (CB) of the normal eccrine sweat gland duct. Blunt microvilli (MV) project into the lumen. The duct in this instance is contained within one cell, with the nucleus (N) to the left. The cytoplasm borders on the lumen and is connected to adjacent cells by numerous desmosomes (D). No cell-to-cell junctions are present in the cuticular border to indicate that more than one cell participates in the formation of this lumen. A zone of small microvilli (MV) are present to the lower right indicating another lumen in partial section. × 11,000.

spaces (Figs. 18 and 19), and as the apposed cell membranes abut on the lumen they are thickened to form terminal bars (Figs. 18 and 19). Blunt microvilli from the apical cytoplasm project into the lumen.

The second type of duct-like structure has a small lumen containing granular material and is lined by a very thin, dense "cuticular border," usually belonging to a single tumor cell. This single cell has a lumen in the midst of its cytoplasm and connects with other duct-like lumens present (Figs. 16 and 17). The "cuticular border" lining such a lumen is a more compact version of that illustrated for the cuboidal cells in Figure 19.

*"Old" Nodule—Present for Many Years*

*Light Microscopy:* The general appearance of this nodule (Fig. 6) is similar to that described

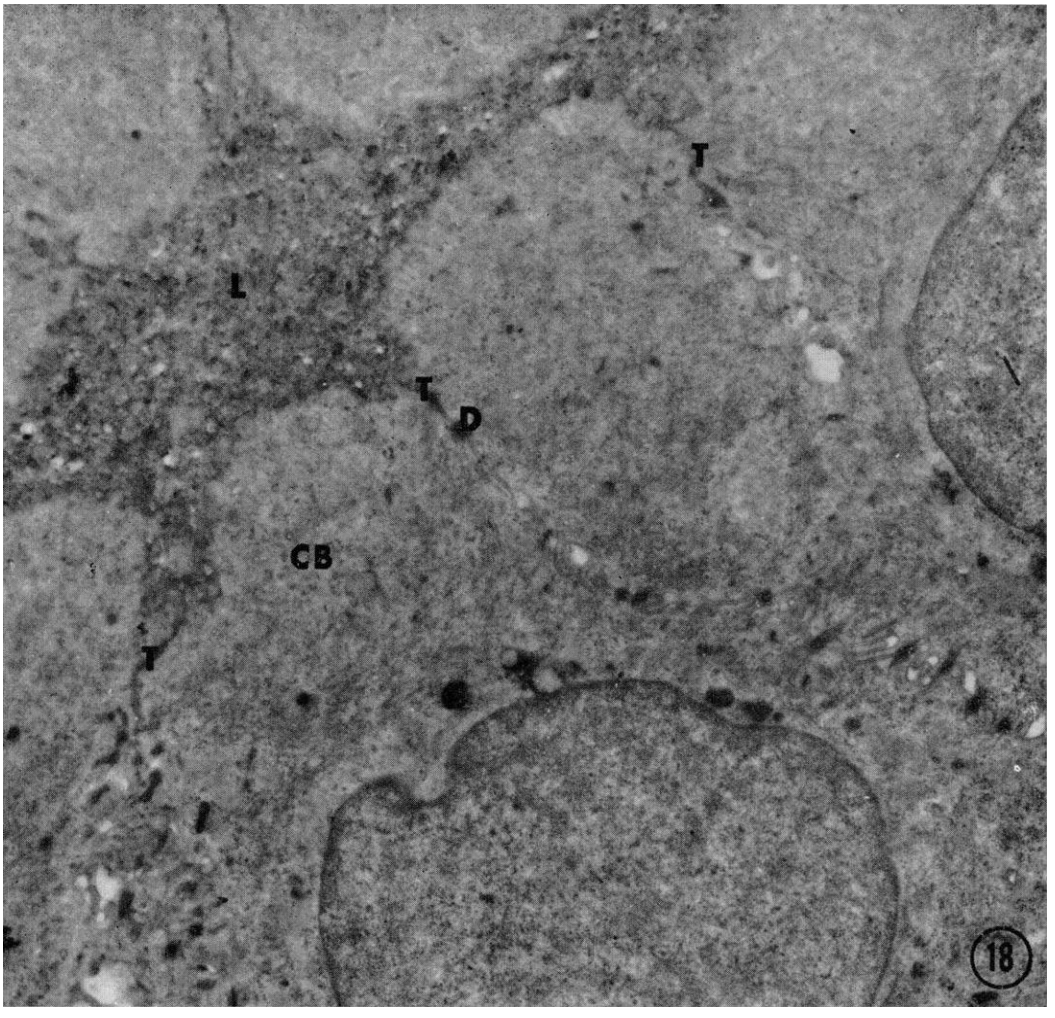


FIG. 18. Higher magnification of duct similar to that in the upper portion of Fig. 16. The lumen (L) contains dense granular material. The apical portion of the surrounding cells is specialized into a cuticular border (CB) containing filamentous material. (See Fig. 19.) Between adjacent cells as they abut on the duct lumen are occasional desmosomes (D), and the apposing cell membranes are thickened into terminal bars (T). × 9,100.

above for the "young" nodule except for one distinct difference. In the "old" nodule the hyalinized basement membrane is much thicker and more compact (Fig. 6), more eosinophilic, and more intensely PAS positive (Fig. 7). Some intralobular hyaline material is also present. In several instances connections can be seen between the hyalinized basement membrane and the intralobular hyaline masses. Both loose and solid patterns of tumor cells are present, and intercellular acid mucopolysaccharide is present, as demonstrated by the colloidal iron technique. Pockets of acid mucopolysaccharide located within the hyalinized basement membrane are not as frequent as in the "young" nodule. Ductlike structures are identical to those described for the "young" nodule.

*Electron Microscopy:* The characteristics of the tumor cells and the general pattern (Fig. 20) are identical to those described for the "young" nodule. The hyalinized basement membrane is a thick, uniform, dense, concentrically laminated mass surrounding the cords of cells (Figs. 20 and 21). In some areas within the basement membrane small clefts are present that may represent embedding artifact. Occasional delicate fingers of cytoplasm from the tumor cells extend into the substance of the hyalinized basement membrane. Intralobular masses of hyaline material are present, and some connect directly with the basement membrane by a delicate

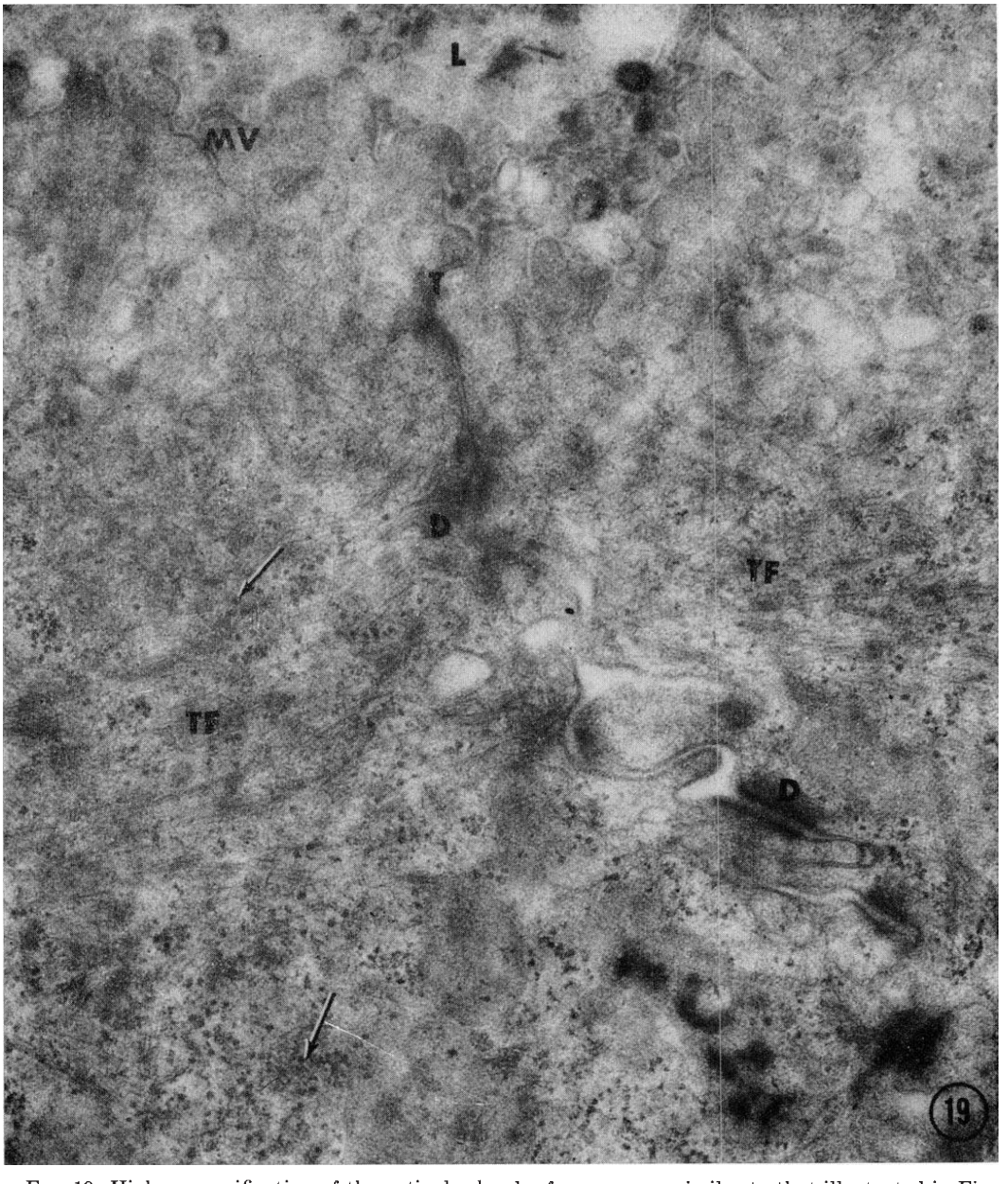


FIG. 19. Higher magnification of the cuticular border from an area similar to that illustrated in Fig. 18. The lumen (L) is at the top of the micrograph. Small blunt microvilli (MV) project into the lumen. The apical cytoplasm of the cells is filled with numerous tonofilaments (TF) and small granules (arrows). The adjacent cells are connected by numerous desmosomes (D), and the luminal edges are joined by terminal bars (T). × 52,600.

stalk (Fig. 20). Duct-like structures (Fig. 21) have a similar ultrastructure to that described for those of the "young" nodule.

*Irradiated Nodule*

*Light Microscopy:* In the irradiated nodule only a skeleton of the original tumor mass is present. The hyalinized basement membrane material (Fig. 8) and only scant fibroblastic cellular elements remain. The basement membrane material is still PAS positive and colloidal iron reactive.

*Electron Microscopy:* Dense zones of hyaline amterial similar to that described previously are

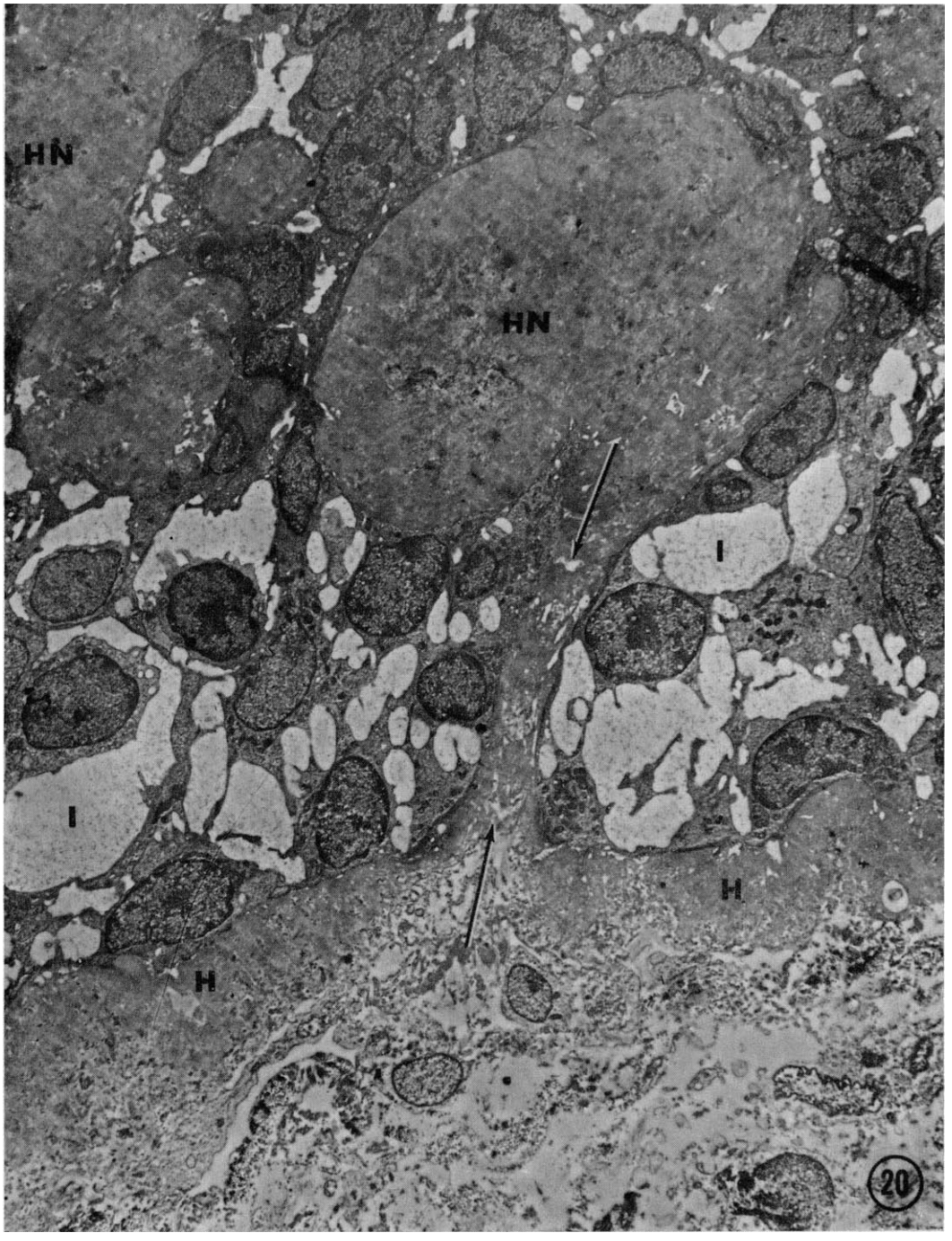


FIG. 20. "Old" nodule. A cord of tumor cells is illustrated containing a predominantly reticular pattern with prominent intercellular spaces (I). The hyalinized basement membrane (H) is very thick, dense, and uniform, and lacks the undulating bands seen in the "young" nodule. Within the hyalinized basement membrane are small clefts, perhaps representing embedding artifact. Nodules of hyaline material (HN) surrounded by tumor cells are present, and the one in the upper center of the electron micrograph is connected to the stroma by a narrow stalk (arrows). × 3,977.

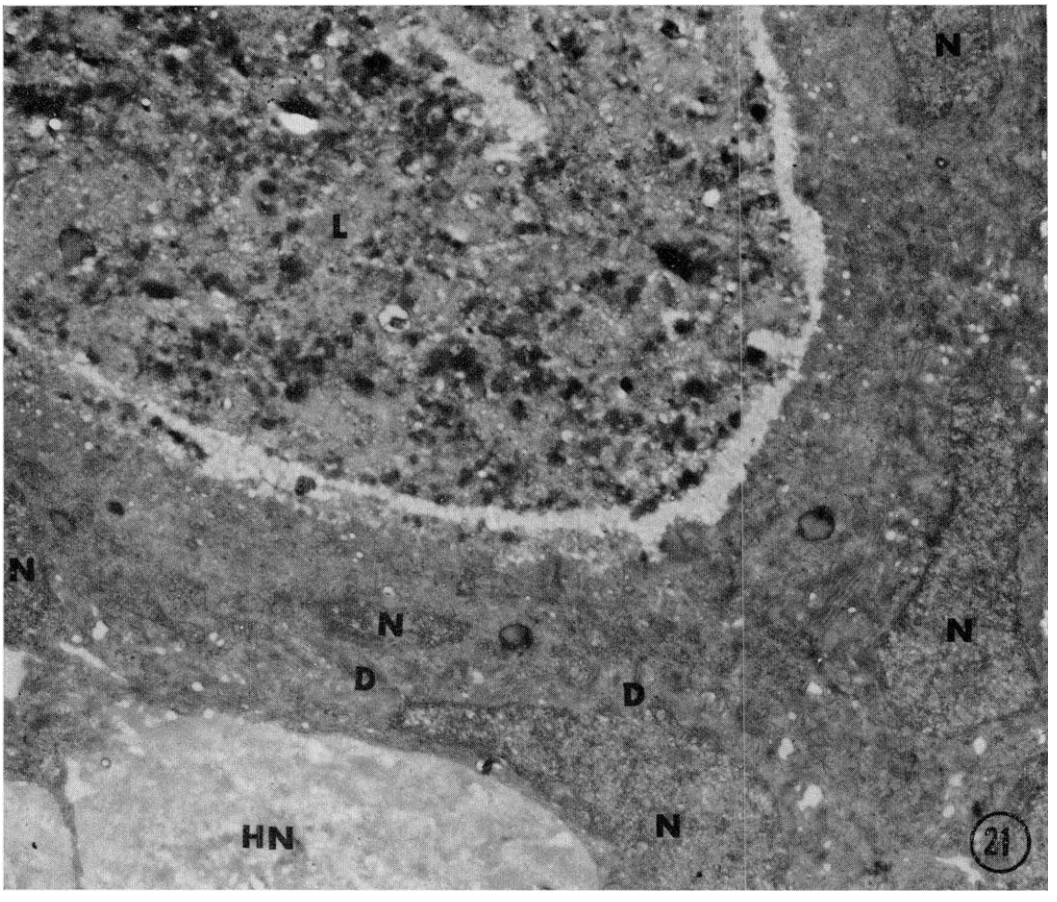


FIG. 21. "Old" nodule. Higher magnification of a duct with a lumen (L) containing dense granular material. This is lined by flattened cells, the nuclei (N) of which can be easily identified. A portion of a hyaline nodule (HN) is present to the lower left. Desmosomes (D) connect adjacent cells. × 11,640.

found in the midst of bundles of collagen, capillaries, and a few fibroblasts. No tumor cells could be identified.

## DISCUSSION

The dermal eccrine cylindroma is an acid mucopolysaccharide-secreting tumor of the skin with many similarities in ultrastructure and histochemical properties to eccrine or apocrine sweat glands. The presence of lakes of acid mucopolysaccharide separating cells within the cords suggests that the tumor cells secrete this material, since no other cells are present to account for its production. Further evidence for secretory activity is provided by the presence of prosecretory and secretory vacuoles within the cytoplasm of the tumor cells and by the association of prosecretory vacuoles with the Golgi apparatus. Mucoid cells (formerly termed dark cells) of the eccrine sweat gland secretory segment (13, 16, 17) and the secretory cells of the apocrine sweat gland (11) normally secrete mucopolysaccharides by means of secretory vacuoles. In the eccrine sweat gland these secretory vacuoles form from prosecretory vacuoles occurring within the membranes of the Golgi apparatus. Thus the secretory structures of the tumor cells are similar to those of eccrine or apocrine sweat glands, but more like the eccrine. The conclusion of Lennox *et al.* (8) is interesting in this connection in that they felt that "a primary skin tumour, with mucin in it, is always of sweat gland origin." Our observations on the mucin content of dermal eccrine cylindroma, coupled with the fact that the normal eccrine sweat gland secretes an acid mucopolysaccharide, certainly support the opinion of Lennox *et al.* (8).

The duct-like structures present within this tumor are strikingly similar to those of the eccrine or apocrine gland (3, 13, 16, 17). The cuticular border of the apical cytoplasm of the luminal cells within the tumor has the same general configuration as the cuticular border of the eccrine duct; that is, it is composed of granules and a network of tonofilaments that insert into the numerous desmosomes joining adjacent ductal cells. The large cuticular borders seen in the tumor resemble more those of the eccrine duct, since the apocrine duct has a very small cuticular border and is hardly demonstrable by light microscopy (11, 12).

The ultrastructure of the dermal cylindroma does not definitely solve the problem of its histogenesis, since it has features that resemble both the eccrine and the apocrine sweat glands. The difference between eccrine and apocrine sweat glands is not as great as has been previously assumed (25), especially since both glands have a cell that secretes mucopolysaccharide (13). Furthermore, eccrine glands are not peculiar to primates, for the glands of the cat foot and toe pads are definitely eccrine in nature and comparable to the eccrine glands of man (16). Thus, the tumor in question may be derived from a cell that has the potentiality of differentiating in the direction of, or mimicking the structure of, sweat glands in general. There is absolutely no evidence that myoepithelial cells are present within the tumor cords, as suggested by Lever (7).

Growth of the tumor is probably attributable to accumulation of hyaline material surrounding the cords of cells. The presence of hyaline material can be explained on the basis of extrusion into the basement membrane of the mucopolysaccharide secreted by the tumor cells. Since these tumors grow slowly, this extrusion must occur at a very slow rate. The separation of the basement membrane from the base of the tumor cells and the undulating pattern (demonstrated by the PAS preparation or by electron microscopy) can also be explained by the extrusion of material into the basement membrane. This growth pattern is repeated, coupled with the formation of new basement membrane along the base of the tumor cells. Eventually the basement membrane material, together with the collagen that inserts into its stromal surface and the extruded mucopolysaccharide, condenses and becomes compact, as seen in the nodule of long duration. Since this growth pattern would result in successive concentric deposits of material around the cords of cells, fragmentation along lines of least resistance seen in methacrylate-embedded tissue gives the appearance of laminated deposits. The relationship of fat (1) in the hyalinized basement membrane to the ultrastructure of the tumor is not known.

The intralobular deposits of hyaline are actually invaginations of the hyalinized basement membrane into the tumor lobule. The frequent observation by both electron and light microscopy that continuity exists between these hyaline nodules and the basement membrane makes the possibility very likely that such continuity exists for all such intralobular deposits of hyaline. The developmental pattern that would explain this appearance could be either excessive growth of a portion of the cord of tumor cells, producing material and extruding it into the stroma, or collapse of the cords of cells following liberation of secretory products into the basement membrane.

In many respects this tumor resembles the eccrine spiradenoma in ultrastructure and histochemical properties (15); in other respects it is quite different. The basic ultrastructure of the cells comprising the two tumors is similar, but cells of the dermal eccrine cylindroma have secretory vacuoles. Hyaline nodules are present in both tumors. In eccrine spiradenoma these appear to be derived from the stroma exclusively, whereas in dermal eccrine cylindroma they are made up of a combination of basement membrane, stroma, and mucopolysaccharide secreted by the tumor cells. The distribution of acid mucopolysaccharide material is also different in the two tumors. The eccrine spiradenoma has acidic mucopolysaccharide only within the hyaline nodules, while the dermal eccrine cylindroma has acid mucopolysaccharide material located in the intercellular spaces within the cords of tumor cells. Both tumors have a similar general architecture comprised of cords and whorls of cells embedded in a loose connective tissue stroma. These similarities suggest a common origin; that is, the eccrine sweat glands.

## SUMMARY

The dermal eccrine cylindroma (turban tumor) is composed of acid mucopolysaccharide-secreting cells that contain secretory vacuoles and resemble sweat gland secretory cells. The acid mucopolysaccharide is secreted into large intercellular

spaces and from here is extruded into the basement membrane, subsequently forming pockets of material. These pockets increase in size and number, and the basement membrane condenses and gradually becomes transformed into the hyalinized basement membrane seen by light microscopy. Present within the cords of tumor cells are duct-like structures similar in staining properties and ultrastructure to the eccrine sweat gland ducts. Dense granules of undetermined nature are also seen in some tumor cells. The tumor cells have never been observed to contain myofilaments.

Based on the similarities between the tumor cells and eccrine sweat glands in terms of acid mucopolysaccharide secretion and ductal structure, the tumor is considered to be, if not derived from, at least mimicking eccrine sweat gland structure.

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## DISCUSSION

Dr. Andrew E. Lorincz, Gainesville, Florida: I would like to make a comment and ask a question relating to the use of the expression "acid mucopolysaccharide" as used by the authors. Acid mucopolysaccharides biochemically are substances that have been well characterized. Materials that histochemically are PAS-positive or stain with colloidal iron are not necessarily acid mucopolysaccharides, although acid mucopolysaccharide complexes as well as other substances react in this manner.

In the condition known as Hurler's syndrome

or gargoylism, where chemically demonstrated excessive amounts of acid mucopolysaccharide have been found in tissues, electron microscopic analyses of liver, do not show the same appearance as the structures considered to be acid mucopolysaccharide aggregated by Dr. Munger and co-workers. Aggregates of mucins and mucoproteins of varying kinds have a very similar appearance to the acid mucopolysaccharide protein complex aggregates on ultrastructural analyses. This has been well demonstrated in the goblet cell mucus-secreting glands of the rat intestine, as well as in liver of individuals with the Hurler syndrome.

My question specifically is, what evidence do the authors have that the material, which they refer to as "acid mucopolysaccharide", is truly acid mucopolysaccharide? Was this verified by chemical isolation? If only histochemical technics were employed, was the material digested by hyaluronidase?

Dr. Bryce L. Munger, (in closing): Dr. Lorincz has brought up a very complicated problem, that is the reliability of histochemical tests for polysaccharides. I think it is sufficient to say, without going to great lengths, that the histochemical identification of mucopolysaccharides, characterized by the battery of technics available, are probably the best of the specific stains available in any histological laboratory department in chemistry and even that may not give the specific mucopolysaccharide stains. The mucopolysaccharide in question is not PAS positive, while the basement membrane *is* PAS positive. It is an acid mucopolysaccharide based on its reaction with colloidal iron in alcian blue. It is not digestible with hyaluronidase and thus is not ground substance.

In the normal eccrine sweat glands such an acid mucopolysaccharide is present, and hexosamine has been chemically isolated from human eccrine sweat. The evidence that there is really an *acid* mucopolysaccharide in the tumor is based strictly upon these specific stains at histologic level. There is no chemical evidence for them to be truly "acid."

With respect to the ultrastructural appearance of the acid mucopolysaccharide, one finds a similarity between various structures. In the case of the Hurler's syndrome, this, I think is the exception.